Utility and Safety of Commercially Available Injection Laryngoplasty Materials in a Rabbit Model

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Summary: Objective. To demonstrate foreign body and chronic inflammatory reaction of commercially available injection materials using the rabbit vocal fold paralysis model.

Study Design. Animal study.

Methods. The left recurrent laryngeal nerve was identified and divided at the tracheoesophageal groove. Amounts (100 μL) of phosphate-buffered saline (PBS), polyacrylamide hydrogel (Aquamid; Ferrosan A/S, Søborg, Denmark), calcium hydroxyapatite (Radiesse; BioForm Medical Inc., San Mateo, CA), or hyaluronic acid derivative (Rofilan; Rofil Medical International, Breda, Netherlands) were injected into the left vocalis muscle. Six months later, the larynx was harvested. Hematoxylin/eosin and Masson trichrome staining were performed to compare inflammatory and foreign body reactions, granuloma development, and relative vocal fold areas among groups.

Results. Compared with the PBS (control) group, the Aquamid, Radiesse, and Rofilan groups exhibited only mild chronic inflammatory reactions that did not significantly differ among groups, or from controls ($P > 0.05$). However, the Aquamid and Radiesse groups exhibited moderate foreign body reactions that were significantly greater than those of controls ($P < 0.05$). No foreign body granuloma formed in any group. All test groups exhibited significant increases in vocal fold areas at 6 months ($P < 0.05$).

Conclusions. Although commercially available injection materials induced more foreign body reactions than a control injection of PBS, no foreign body granuloma developed and the augmented vocal fold area was maintained until 6 months after injection.

Key Words: Injection laryngoplasty–Vocal fold–Foreign body.

INTRODUCTION

Recently, injection laryngoplasty has regained popularity in the management of unilateral glottal insufficiency because the technique is easy to use and less invasive than conventional medialization thyroplasty.[1,2] With advances in biotechnology, several excellent injection materials have become available, including Artecoll (20% [vol] polymethyl methacrylate (PMMA) microspheres + 80% [vol] bovine collagen; Rofil Medical International, Breda, The Netherlands); Radiesse (25% calcium hydroxyapatite + 75% carboxymethylcellulose; BioForm Medical Inc., San Mateo, CA); Aquamid (2.5% cross-linked polyacrylamide polymer and 97.5% water, Ferrosan A/S, Søborg, Denmark); and a hyaluronic acid derivative (Rofilan; Rofil Medical International, Breda, The Netherlands).[3-6] However, the only vocal fold injection material approved by the food and drug administrations of the United States and Korea is Radiesse.[7]

Most vocal fold injection materials used today are approved as facial soft tissue fillers. Injection of such fillers into the vocal fold is an off-label use for which safety data are not available.[8] Therefore, it is essential to verify the safety of such materials when injected into the vocal folds. We investigated the safety and utility of commercial injection materials in a rabbit vocal fold paralysis model.

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MATERIALS AND METHODS

Animals

Twenty adult female conditioned laboratory New Zealand rabbits were used in this study (body weights: 2.5–3 kg). All surgical and animal care procedures were performed in accordance with our institutional policy on the care of experimental animals (Animal IRB approval No.: SCHBC_animal_20 1010).

The rabbits were divided into four groups of six rabbits each, by the type of material injected. Amounts (100 μL) of phosphate-buffered saline (PBS; control group), polyacrylamide hydrogel (Aquamid; Ferrosan A/S), calcium hydroxyapatite (Radiesse; Bioform Medical Inc.), or hyaluronic acid derivative (Rofilan; Rofil Medical International) were injected into the left paralyzed vocalis muscle on the side of recurrent laryngeal nerve (RLN) transection.

Surgical procedures

Rabbits were anesthetized by intramuscular injection of a mixture of Rompun (xylazine hydrochloride; Bayer, Pittsburgh, PA) at 5 mg/kg and Zoletil 50 (tiletamine with zolazepam; Virbac, Carros, France) at 10 mg/kg. The left RLN was identified and divided at the tracheoesophageal groove to create a model of rabbit vocal fold paralysis. A 7.5-cm-long nasal speculum was introduced through the oral cavity to expose the vocal folds. A 30° angled 2.7 mm × 30 cm telescope (Karl Storz Co., Tuttlingen, Germany) was passed through the speculum to maximize visualization during injection. Six months after injection, each larynx was harvested and sent to our pathology department. All surgical procedures and section preparations were performed by a single surgeon (S.W.L.)

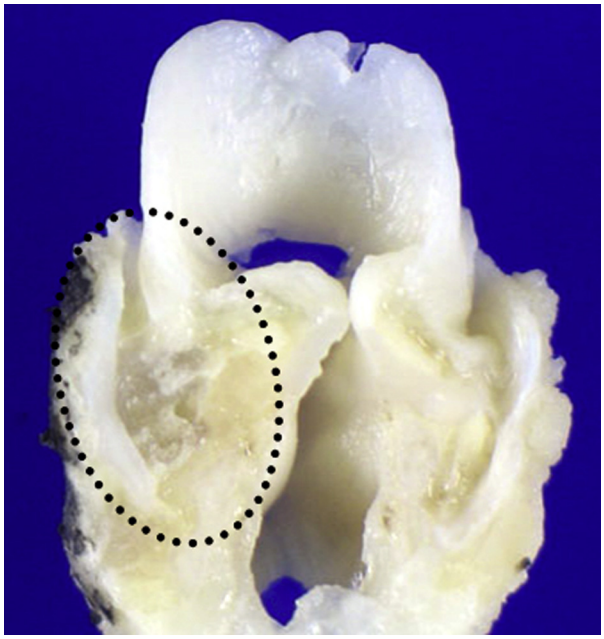

FIGURE 1. A gross laryngeal coronal section showing that grafted polyacrylamide hydrogel (Aquamid) maintained vocal fold volume compared with that of the contralateral side (circle).

Histopathologic analysis

Whole larynges were subjected to coronal sectioning, and slides that included the centers of the membranous vocal folds were histologically analyzed. Laryngeal tissues were fixed in 10% formaldehyde and then embedded in paraffin. Six-micrometer-thick sections were prepared for histological examination. Hematoxylin eosin (HE) staining was used to assess the extent of chronic inflammatory reactions based on infiltration of lymphocyte and to detect foreign body reactions based on infiltration of macrophages and multinucleated giant cells. Masson's trichrome staining was used to compare vocal fold areas among groups[9] (Figures 1 and 2).

The extent of a chronic inflammatory reaction was graded from 0 (absent) to 3 (severe; intense infiltration) by a single experienced pathologist (H.K.K.) blinded to experimental information.

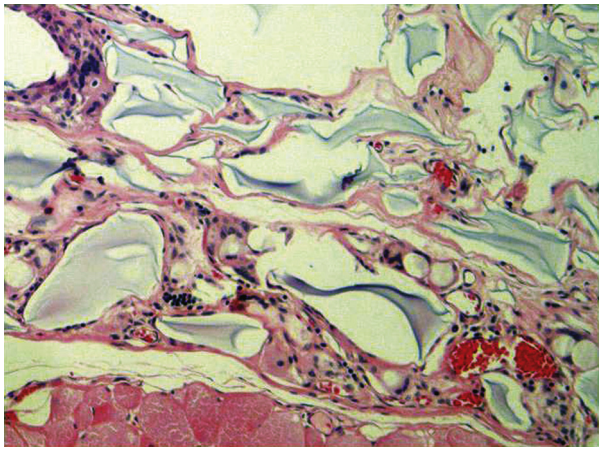

FIGURE 3. A grade 2 (moderate) foreign body reaction to a polyacrylamide hydrogel (Aquamid). A macrophage and a multinucleated giant cell may be noted around the Aquamid injection site 6 months after injection (HE stain, ×100).

Under high-power magnification (×100), the extent of lymphocytic infiltration around injection materials was assessed on three different slides, and these figures were averaged and used in comparisons.[10,11] The extent of a foreign body reaction was also classified from grade 0 to 3 in a same manner (Figure 3).

Image analysis

Each vocal fold was examined at a magnification of ×10, and images were captured using a Nikon Eclipse 80i microscope (Nikon Corporation, Tokyo, Japan) fitted with a Jenoptik color camera (ProgRes C10 Plus; Jenoptik, Jena, Germany). *ImageJ* (ImageJ 1.43u; National Institutes of Health, Bethesda, MD) and *Adobe Photoshop image analysis software* (Adobe Systems Inc., San Jose, CA) were used to measure the areas of vocal folds.[9] The ratio of pixels in the injection area relative to the total number of pixels in the same area of the contralateral normal vocal fold was the vocal fold square ratio (Figure 4).

To minimize measurement bias, all measurements were conducted by one pathologist blinded to group data. The Mann-Whitney *U* test was used to compare control and experimental group, and $P < 0.05$ was considered statistically significant.

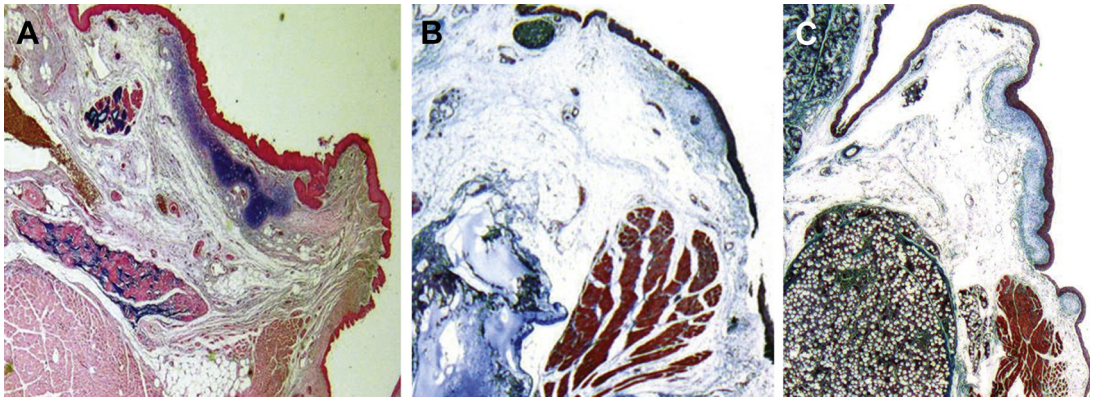

FIGURE 2. A representative photographs of three fillers in the left vocal fold at 6 months after injection (Masson trichrome stain, ×100). **A.** Hyaluronic acid derivative. **B.** Polyacrylamide hydrogel. **C.** Calcium hydroxyapatite.